Estonian University of Life Sciences

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MEAT INSPECTION FINDINGS INDICATING NECROBACILLOSIS IN FINNISH REINDEER DURING 2004-2016

NEKROBATSILLOOSILE VIITAVAD LIHAINSPEKTSIOONI LEIUD SOOME PÕHJAPÕTRADEL AASTATEL 2004-2016

Final Thesis in Veterinary Medicine

Curriculum in Veterinary Medicine

Supervisors: Sauli Laaksonen, DVM, PhD. Ad.prof

Professor Arvo Viltrop

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ABSTRACT

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Necrobacillosis is an important and common disease of reindeer that hasn't been studied profoundly in Finland before. It used to occur as a foot form called "slubbo" in the past, but nowadays the gastrointestinal form causing abscesses and inflammation in the mouth and inner organs is more common and often leads to death or condemnation of the carcass or parts of it. In this study, the case of inflammation indicating necrobacillosis (IIN) is classified a registered case of abscess, peritonitis, pleuritis or pneumonia that has caused the condemnation of the whole or parts of the carcass. The meat inspection data originated from official meat inspection documents from the years 2004-2016 made by veterinarians from all the approved reindeer abattoirs of Finland. This data was analyzed using Excel 2016 and STATA 14.0 to study frequency of IIN-s. The incidence rate ratios were calculated to compare the frequencies in different areas and years. The average frequency of IIN-s in slaughter batches in the whole in Finnish reindeer herding area was 3.5% during 2004-2016. The risk for IIN is higher in the Southern areas. There wasn't clear association between weather conditions and occurrence of IIN. Still, summers preceding high risk years were warmer than the average of study period. This was the first attempt to study the occurrence of necrobacillosis in Finland and showed that this disease syndrome needs further investigation and research.

Keywords: necrobacillosis, frequency, risk factor, area, weather

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Juhendajad: ad. prof. Sauli Laaksonen, prof. Arvo Viltrop
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<tr><td colspan="4">Nekrobatsilloos on majanduslikult tähtis põhjapõtrade haigus, mida ei ole Soomes veel uuritud. Minevikus esines nekrobatsilloos tavaliselt jalavormina (nimetusega “slubbo”), aga tänapäeval on seedetrakti vorm tavalisem ja see lõpeb tihti looma surmaga või on selle tagajärjeks terve või osaline lihakeha praakimine. Selles uuringus on nekrobatsilloosile viitav põletikuline muutus (IIN) määratletud kui tapapõhjapõtradel tuvastatud pleuriit, peritoniit, pneumoonia või abtsess, mille tulemusena on lihakeha tervikuna või osaliselt praagitud. Lihainspektsiooni andmed pärinesid loomaarstide poolt koostatud ametlikest lihainspektsiooni dokumentidest igast Soome ametlikust põhjapõtrade tapamajast. Andmed analüüsiti kasutades Excel 2016 ja STATA 14.0 IIN sageduse ja nende suhtelise sageduse (IRR- *incidence rate ratio*) leidmiseks. IRR abil võrreldi INN sageduse erinevust eri piirkondades ja aastatel. Nekrobatsilloosile viitavate lihainspektsiooni leidude esinemissagedus tapaloomade partiis oli kogu põhjapõtrade pidamise alal aastatel 2004-2016 keskmiselt 3,5%.IIN esinemise sagedus oli suurem lõuna piirkondades. Ilmastikutingimuste ja IIN esinemissageduse vahel ei olnud väga selget seost, kuid kõrgema riski aastatele eelnevad suved olid soojemad kui uuringuperioodi keskmine. See uuring oli esimene kord kui nekrobatsilloosi levikut Soome põhjapõtradel uuriti ja näitas et haigus vajab rohkemat ja täpsemat uurimist.</td></tr>
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INTRODUCTION

Necrobacillosis is an infectious disease of several mammals, including ruminants, horses and human, caused by the family of Fusobacteriaceae, mostly by *Fusobacterium necrophorum.*

Necrobacillosis has been described in the past as a feared disease of reindeer. Horne (1897) and Qvigstad (1961) (reviewed by Handeland *et al.* 2009) mention digital necrobacillosis being the most serious health problem in Norwegian reindeer herding in the 1800s and early 1900s, probably caused by intensive reindeer herding at that time. It was also common and devastating disease and caused several outbreaks in the history of Finnish reindeer herding (Laaksonen 2016). Horne (1897), reviewed by Handeland *et al.* 2009, and Turi (1910), reviewed by Rockborn *et al.* 1990, report a disease producing ulcerations in the upper respiratory and oral mucosa of reindeer. Horne (1897) and Qvigstad (1941), reviewed by Handeland *et al.* 2009, and Skjenneberg and Slagsvold (1968), reviewed by Wikström 2014, also suggest that there's a connection with outbreaks of oral and generalized necrobacillosis. Nikolaevski (1961) has also described a similar disease complicated by oral necrobacillosis in the USSR (reviewed by Rockborn *et al.* 1990).

Necrobacillosis can affect different parts of the body, but usually results in inflammation in the oral, thoracic and peritoneal cavity and abscesses in inner organs and around body (Hirvelä-Koski *et al.* 2017). Laaksonen (2016) suggests, that reindeer necrobacillosis can be considered the most common and important bacterial disease all around the Finnish reindeer herding area. Also, recent report by Hirvelä-Koski *et al*. (2017) revealed, that 45% of the reindeer, that had their samples sent to Finnish Food Safety Authority EVIRA because of disease, died of necrobacillosis, being the most common cause of mortality. It is suggested that warm summers result in higher incidence of necrobacillosis (Woodbury and Chirino-Trejo 2004, Laaksonen 2016, Nikolaevskii 1961) and supplementary feeding and corralling are predisposing factors (Rehbinder and Nikander 1999, Laaksonen 2016, Tadepalli *et al.* 2009, Wikström *et al.* 2014).

Necrobacillosis is a very important disease syndrome that has not yet been studied profoundly in reindeer. This thesis studies the frequency of necrobacillosis in Finnish

reindeer based on post mortem findings indicating necrobacillosis during meat inspections in 2004-2016. The impact of areal and climatic risk factors affecting the occurrence of the disease are also studied. This is done through statistical analysis, using Finnish Meteorological Institute's weather data for the evaluation of climatic effects.

I want to thank Sauli Laaksonen and the project "Reindeer health in changing environment" (Makera) for all the data provided on pathological findings and Finnish Meteorological Institute for the weather data. I also want to thank my supervisors ad.prof. Sauli Laaksonen and professor Arvo Viltrop for all the help with this work and I'm especially grateful to professor Toomas Orro for spending so many hours helping me with the statistical analysis.

1. LITERATURE REVIEW

1.1. Reindeer husbandry in Finland

The area of reindeer husbandry covers approximately 36% of Finland and is mainly located in Lapland, in addition to a smaller area in Northern Ostrobothnia and Kainuu region (Reindeer Herders' Association website, visited 1 April 2018). This area is divided to 54 smaller regions called reindeer herding co-operatives. Reindeer husbandry is based on Eurasian semi-domesticated reindeer (*Rangifer tarandus tarandus*), meat being the most important product (Laaksonen *et al.* 2017). Reindeer are allowed to range free in the co-operatives herding area. They are usually gathered for round-ups twice a year – in June-July for ear marking of calves and in September-January round-ups to select reindeer for slaughter and breeding (Reindeer Herders' Association website, visited 1 April 2018). Most of reindeer receive supplementary feed either in the field or in corals for some winter months (Laaksonen *et al.* 2017). In the year 2000-2001, over 40% of the reindeer were corralled at least for 90 days throughout the winter, emphasis being on the southern areas with 71% of the animals corralled (Maijala and Nieminen 2004). Field feeding was most common in the middle of the reindeer herding area (Maijala and Nieminen 2004).

In the year 2015 there were about 194 000 reindeer in the reindeer management area after slaughter. The estimated number of the heads in the autumn is about 270 00-300 000 and about one third of them are slaughtered annually (Laaksonen 2010). A big majority of slaughtered animals are calves from five to nine months (Laaksonen 2010). Approximately 74% of the animals are slaughtered in EU approved slaughterhouses and the rest on the field for own consumption or direct marketing (Laaksonen *et al.* 2017). In 2012/2013 approximately 90 000 reindeer were slaughtered, producing about 2 million kilograms of meat (Reindeer Herders' Association website, visited 1 April 2018).

1.2. Causing agent

Fusobacterium necrophorum is a gram-negative, obligate anaerobe. According to Handeland *et al.* (2010), it can also be found in a healthy animal, especially in rumen. It produces toxins, mainly leuko- and endotoxins that can destroy tissues, often leading to

secondary bacterial infections caused by, e.g. *Actinobacillus*, *Actinomyces* and *Corynebacterium*. Smith *et al.* (1989) also suggest, that infectivity of *F. necrophorum* is enhanced by the presence of these bacteria. According to Woodbury and Chirino-Trejo (2004), volatile fatty acids produced by *F. necrophorum* lower the function of leucocytes, and leucocidins of the bacteria destroy many of them arriving at the lesion site. *Fusobacterium* cannot penetrate intact skin. Entry and infection of the tissues occurs through cuts or abrasions of the skin or mucous membranes lining the mouth or rumen contaminated by food, water or soil containing *F. necrophorum* bacteria (Woodbury and Chirino-Trejo 2004).

1.3. Clinical signs and post mortem findings

Fusobacterium necrophorum can cause inflammation in hooves (foot rot), mouth, tongue and head area, gastrointestinal tract and peritoneum, lungs and pleura and sometimes in genitals and various tissues. The disease can be local or generalized. Symptoms vary depending on the site of the inflammation. In the head area increased salivation, anorexia and difficulties with chewing can be seen (Laaksonen 2016). When organs or chest cavity are affected, the reindeer can be asymptomatic for quite long leading to sudden morbidity due to the chronic inflammation process. Sudden deaths can be caused by the rapid release of tissue toxins into the blood stream (Laaksonen 2016).

Post mortem findings usually include necrotic ulcers and abscesses in the oral cavity, especially in mucous membranes of the throat. If the bacterium is spread through the blood circulation, abscesses can be found in various inner organs, often in liver. Lesions in rumen are typically yellow, raised necrotic areas or abscesses. According to Woodbury and Chirino (2004), in the case of perforation, the disease can spread into the abdominal cavity and cause peritonitis. Pleuritis is also a common finding (Laaksonen 2016).

Figure 1. An abscess under the tongue caused by necrobacillosis. (Photo: Sauli Laaksonen)

Regarding post mortem findings, inflammation of the mouth is a common finding in young animals with swelling of head and jaws. Exudative and necrotic inflammation in the mouth, including tongue and gums are typical findings (Laaksonen 2016) (Figure 1). *F. necrophorum* infections have a typical smell. The inflammation often spreads to peritoneum or lungs (Figures 2 and 3). The blood circulation can take the bacterium into the liver, lungs or brain causing abscesses there. It also has an ability to spread into bones (Laaksonen 2016).

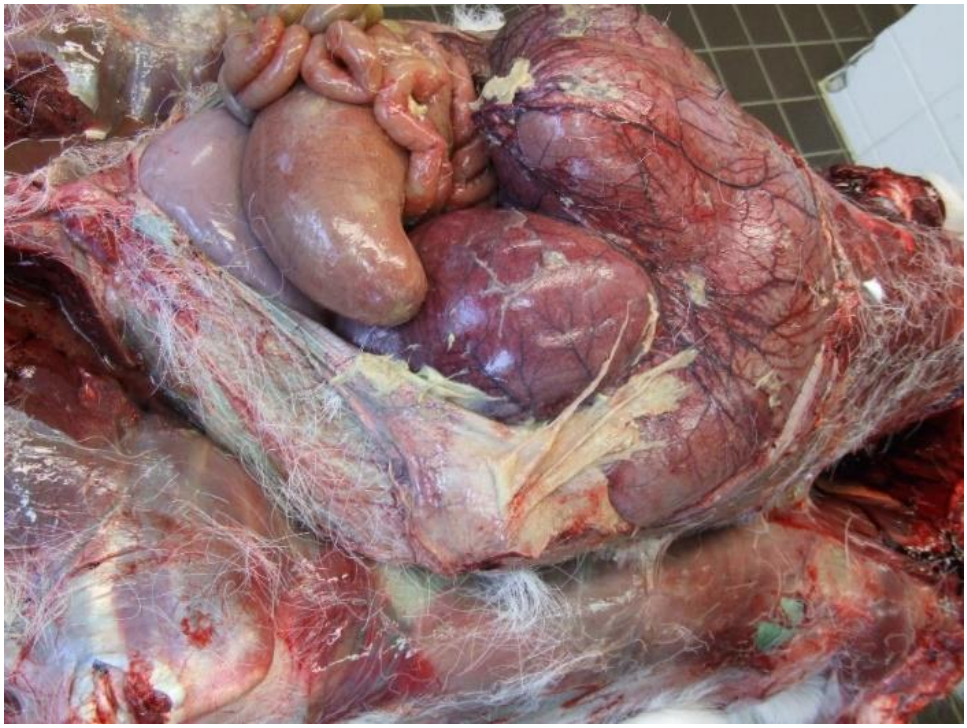


Figure 2. Peritonitis caused by necrobacillosis. (Photo: Sauli Laaksonen)

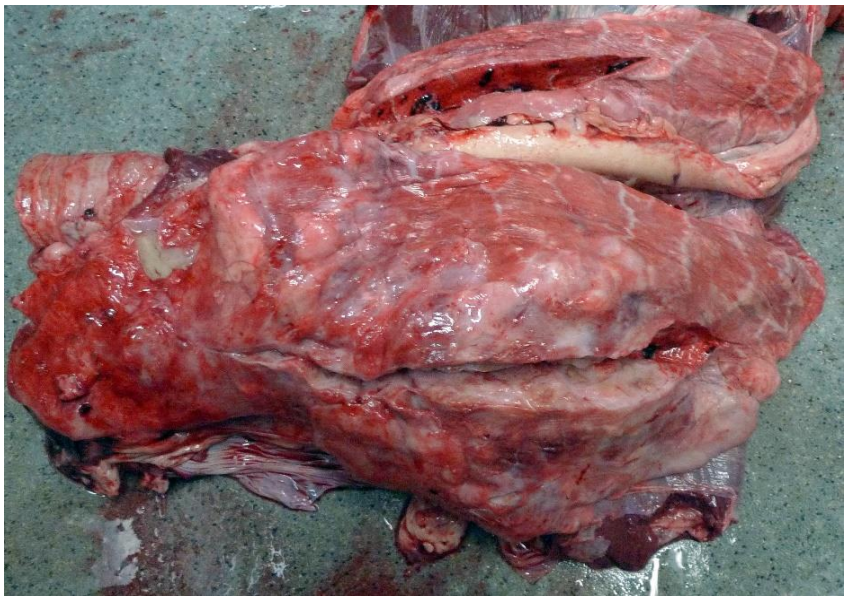


Figure 3. Pneumonia caused by necrobacillosis. (Photo: Sauli Laaksonen)

1.4. Diagnostics

Necrobacillosis is often diagnosed based on the clinical signs and history (Woodbury and Chirino-Trejo 2004). According to Woodbury and Chirino-Trejo (2004), *F. necrophorum* is difficult to culture as it requires specific anaerobic swabs and selective, pre-reduced culture media. There are also a serum ELISA test and a fluorescent antibody test. Handeland *et al.* (2010) established molecular methods to detect the pathogen in reindeer. They also reported that histopathologic lesions of the foot caused by *F. necrophorum* consisted of ulceration of the skin with underlying necrosis penetrating deep into the dermis and subcutis. The necrotic center of ulcers was bordered by a zone of oedema and infiltration of neutrophils, macrophages and lymphocytes, surrounded by connective or granulation tissue. Vasculitis and extensive thrombosis were present in bordering areas. Tongue lesions had ulcers with necrotic margins and peripheral inflammation. Elongate, filamentous gram-negative bacteria were seen mainly at the periphery or the base of the lesions. Regional lymph nodes were enlarged and had changes of hyperplasia and oedema. Hepatic lesions consisted of disseminated foci of peracute liquifactive necrosis with minimal cellular response and foci of acute suppurative and necrotizing hepatitis.

1.5. Treatment

According to Tadepalli *et al.* (2009), *F. necrophorum* is susceptible to β-lactam antibiotics (e.g. penicillins and cephalosporins), tetracyclines (e.g. oxytetracycline), macrolides (e.g. erythromycin and tylosin), lincomycins (clindamycin and lincomycin), chloramphenicol and some other antibiotics, for example ipronidazole. It is insensitive to aminoglycosides, for example gentamycin and streptomycin, which are less active against anaerobes in general. It is also not sensitive to ionophore and peptide antibiotics.

Pietsch *et al.* (1999) reported, that a prophylactic treatment with oxytetracycline during summer handling didn't affect the post-handling survival of reindeer calves. They mentioned that handling stress should be brought to minimum to avoid any damage it can cause, including susceptibility to necrobacillosis.

Woodbury and Chirino-Trejo (2004) suggest that for white-tailed deer, procaine penicillin is an effective but impractical treatment because of somewhat poor penetration and short half-life. Instead, sulfatrimetoprim combinations and tetracyclines are used with better success. They also reported that florfenicol is a good option. Still, prevention - minimizing stress factors and taking care of good hygiene - is much more effective.

In reindeer, the treatment of necrobacillosis includes the application of some of the numerous sulfonamides and antibiotic preparations often combined with surgery, removal of necrotic tissue and pus and applications of antiseptic solutions in local abscesses (Nikolaevskii 1961). In Finland, the reindeer showing symptoms of necrobacillosis are routinely treated with penicillins (or oxytetracycline) by veterinarians with moderate good results provided that the disease has been discovered at the early stage (Laaksonen 2016).

There is also a report of a vaccine against necrobacillosis. According to Mel'nik *et al.* (2009), it decreased the death rate from 53.7% to 17% in nine years. In addition to preventing from falling sick, it also helps the healing of already diseased reindeer. There are no other publications that confirm the efficiency of any vaccine.

1.6. Risk factors

The drivers of occurrence of necrobacillosis in reindeer are not well known. Environmental and management factors, like stress caused by heat, cold, overcrowding or poor nutrition, certainly play a large role in the development of suitable disease conditions (Haigh and Hudson 1993, reviewed by Woodbury and Chirino-Trejo 2004).

Supplementary feeding is supposed to be a significant predisposing factor for the necrobacillosis of reindeer. Coarse feed can make small wounds in the mouth and carbohydrate rich feed can lead to acidosis that causes wounds in the rumen (Rehbinder and Nikander 1999, Laaksonen 2016). Tadepalli *et al.* (2009) suggest that grain-fed cattle had 10-fold higher concentrations of *F. necrophorum* than animals on forage-based diet. Intensive use of reindeer without adequate rest and feeding can lead to necrobacillosis and reindeer worn out during winter show a high rate of necrobacillosis in summer (Nikolaevskii 1961). Also, according to Rehbinder (1982), stress due to handling, transport etc., can cause hemorrhages in rumen, opening the port for the bacterium to enter the circulation. Lesions on mucous membranes can also be caused by other pathogens like poxviruses (orf, pseudocowpox) and herpes viruses leading to secondary infections by necrobacillosis (Rockborn *et al.* 1990, Hautaniemi 2012).

Smith and Thornton (1993) suggest that fecal excretion is probably the primary source of the bacterium with the disturbance of the normal microbiota leading to increased multiplication and excretion. Stress is a predisposing factor and big, overcrowded populations are more prone to infection. Ågren *et al.* (2014) describe an outbreak of oral necrobacillosis in reindeer calves, which was caused by unhygienic feeding conditions due to overcrowding. Because of teething, calves had gingival lesions which were the port of entry for the bacterium. In the herd, five percent of the calves died due to starvation or internal lesions. Pietsch *et al.* (1999) also suggest, that stress due to handling of the reindeer calves may predispose the onset of necrobacillosis.

It is also suggested that the bacterium might be spread by bloodsucking insects indicating that summers with a lot of hematophagous insects can lead to higher incidence of the disease (Nikolaevskii 1961). According to Zhigunov (1961), insect harassment especially caused by *Oestridae* flies (warble fly *Hypoderma tarandi* and throat bot *Cephenemyia*

trombe) contributes considerably to outbreaks of necrobacillosis (Nikolaevskii 1961, Saval'ev 1961). It is also reported that the damage caused by warble fly or throat bot larvae can predispose the development of necrobacillosis in its developing site in subcutaneous tissue or on the mucous membranes of the pharynx (Nikolaevskii 1961)

Necrobacillosis occurs during all the seasons and is more common in calves and lactating females (Laaksonen 2016). It is suggested that hot summers are a predisposing factor (Nikolaevskii 1961, Laaksonen, 2016). Mel'nik *et al.* (2009) suggest that adult reindeer are more likely to be diseased, but reindeer calves have higher mortality. They also describe the disease being more active on tundra than coastal areas and forest. It is also suggested, that muddy and wet camping grounds are dangerous, especially if they are used for long periods (Nikolaevskii 1961).

1.7. Necrobacillosis in cervids

Necrobacillosis is present in several cervid species, e.g. red deer, white-tailed deer, wapiti and North American elk. Edwards *et al.* (2001) describe an outbreak of necrobacillosis in captive wild-caught pronghorns, which killed almost the whole population. The outbreak started after two days of heavy raining with primary pododermatitis, necrotic stomatitis, or sometimes both, which led to septicemia causing lesions in rumen, liver and lungs. In 60 days after the first deaths, only two animals were left, and they were euthanized because of severe pododermatitis. *F. necrophorum* was cultured from most of the lesions.

According to Haigh *et al.* (2005) necrobacillosis is the number one cause of morbidity and mortality in farmed white-tailed deer fawns, and the second in adults, and is probably the most economically important bacterial infectious disease in farmed deer. In farmed white-tailed deer, necrobacillosis causes lumpy jaw and fatal outbreaks of systemic diseases in calves. Systemic disease leading to rapid death is common in fawns. Lesions in the mouth and the pharynx are often deep and can cause abscesses in lungs if bacteria are sucked into the lungs. The interdigital form occurs in deer, while necrotic rumenitis and liver abscesses are rare (Woodbury and Chirino-Trejo 2004).

1.8. Necrobacillosis in reindeer

Laaksonen (2016) suggests, that necrobacillosis is the most common cause of death for reindeer in these days. Nowadays the foot form is rare but other, e.g. oral and gastrointestinal tract disease, are very common. Wikström (2014) found in his study, that necrobacillosis was the primary infectious cause of death of calves born in corrals. He suggests that keeping females in corrals during calving might increase the risk of the death of calves. Again, animal density and hygiene around feeding sites were the most important risk factors for the outbreaks of infectious diseases. According to Mel'nik (2009), reindeer is the most susceptible to necrobacillosis among all domestic animals, being the primary cause of the decrease in reindeer populations in Russia.

Necrobacillosis causes serious economic damage to Northern reindeer herding making up to 30% of total estimated damage. The disease has been recorded among reindeer in all their habitats and the sickness and death rates are 2 to 3 times higher in continental tundra compared to coastal zone. The most problematic places are in the tundra zone where the morbidity is 51.2%, of which mortality 54%. (Mel'nik *et al.* 2009).

In the winter of 1992-1993 there was an outbreak of a contagious disease in Finland causing erosions, papules, pustules and ulcers in the mouth of reindeer. In the outbreak, approximately 400 reindeer died and about 2800 had clinical signs (Oksanen 1993, Tikkanen *et al.* 2004). The main causing agent was Orf virus but in bacteriological studies also *F. necrophorum* among several others were isolated as a secondary infection (Oksanen 1993). Sporadical outbreaks of the "mouth disease" often connected with osteomyelitis of the jaw have been reported ever since in the southern and middle Finnish reindeer herding area (Laaksonen 2016).

Hirvelä-Koski (1999) found out the frequency of the so called "mouth disease", the diagnosis of which was based on clinical signs and post mortem findings typical for necrobacillosis, being 0.4% during the year 1998-1999. The mouth disease was prevalent in all co-operatives where the number of reindeer kept in corrals was over 50%, but not in any where corralling percentage was under 30. The difference was statistically significant and the risk factor for outbreaks seemed to be supplementary feeding with e.g. coarse or frozen feed that damaged the mucous membranes of the mouth. The frequency of the

“mouth disease” didn’t affect next autumn’s number of condemned carcasses or parts of it, but cachexia and abscesses were more often the reason for condemnation in areas where mouth disease was prevalent.

2. AIMS OF THE STUDY

The aims of this study were

1. to estimate the frequency of inflammations indicating necrobacillosis in reindeer in Finland on the basis of the official meat inspection data from the years 2004-2016.
2. to describe the areal distribution of inflammations indicating necrobacillosis in reindeer in Finland
3. to assess the dependence of occurrence of inflammations indicating necrobacillosis in reindeer in Finland from climatic conditions

3. MATERIALS AND METHODS

The meat inspection data of slaughter reindeer were collected from official meat inspection documents of meat inspection veterinarians (see supplementary information file) from all the accepted reindeer abattoirs of Finland, from the slaughter seasons (autumn and early winter) 2004-2005 to 2015-2016. The meat inspection and hygiene control in abattoirs was done by veterinarians who work under the control of Regional State Administrative Agencies of Lapland. The total number of inspected reindeer over the study period was 663585. The animals were slaughtered in 4181 slaughter batches. A slaughter batch represents a group of reindeer from certain area sent together for slaughter at certain time point. The slaughter batch may contain animals from different herds and owners. The epidemiological unit of the study was a 'slaughter batch'. The dataset of 4181 slaughter batches containing information on geographical origin of the batch, date of slaughter, number of animals in a batch, type and number of pathologies in animals of a batch was composed in Microsoft Excel for further analysis.

3.1. Case definition

The case of inflammation indicating necrobacillosis (further referred as IIN-case) was defined as one of the following pathologies recorded in a slaughtered animal: peritonitis, pleuritis, pneumonia or abscesses.

The frequency of IIN-s was calculated for every slaughter batch as number of IIN cases per number of animals in slaughter batch and the frequency of IIN-s in slaughter batches was used as an outcome variable in further analysis.

For comparisons the average frequencies of IIN-s in slaughter batches of comparison groups were used. Also, median frequencies of IIN in batches of comparison groups were found. Because one individual reindeer may have had several IIN cases, e.g. peritonitis and abscesses, in some batches the frequency exceeded 100%. The frequency in these batches was considered as 100%.

3.2. Weather information and reindeer herding area

The weather data from years 2004-2016 was obtained from Finnish Meteorological Institute. The data includes mean monthly temperatures and precipitation in three different weather stations, located in Utsjoki, Sodankylä and Kuusamo, representing the whole Finnish reindeer herding area. Mean summer temperatures were calculated as the mean of June, July and August's mean temperatures of all three weather stations. Winter temperatures include the mean temperatures of December, January, February and March. The same method was used to get the mean precipitation for summer and winter months. Summer months were considered to be of most importance for this study, because most of the slaughtered animals are calves who only live over summer.

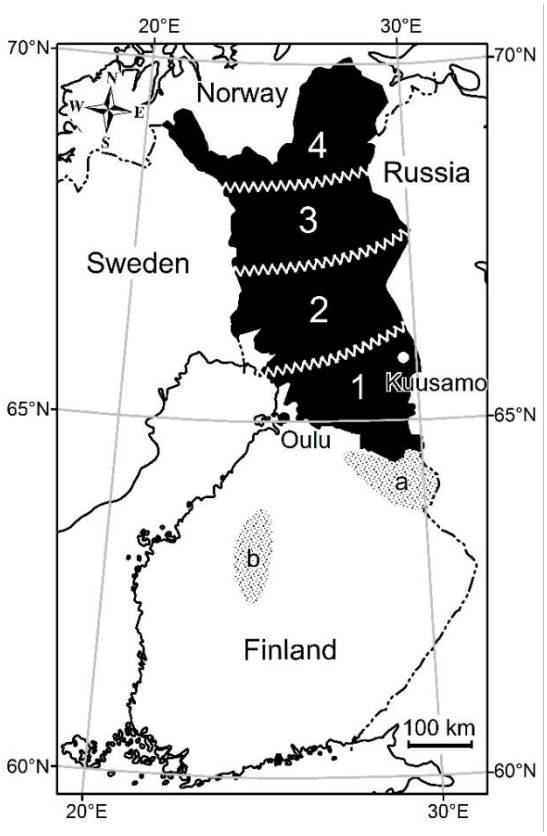


Figure 4. The division of the Finnish reindeer herding area into four areas from South to North.

The Finnish reindeer herding area was divided to four areas according to their geographical position from South to North, number 1 being the Southernmost and number 4 the Northernmost (Figure 4). Areas 1 and 2 form the Southern reindeer herding area and areas 3 and 4 the area specially intended for reindeer herding.

3.3. Statistical analysis

The average and median frequency of IIN-s was calculated for each study year, from four different areas. In this study, years are defined as a reindeer herding year (from the 1st of June to 31st of May). The descriptive statistics was performed with Microsoft Excel 2016.

The risk factor analysis was performed with STATA 14.0 (Stata Corporation, TX, USA) using a random effect negative binomial model where frequencies of IIN in slaughter batches were as outcome variable. This model was chosen because values followed negative binomial distribution. Slaughter month was included as random factor for accounting clustering. Negative binomial model calculates IRR (incidence rate ratio) values between comparing groups. IRR expresses the relative frequency of observations of IIN-cases in different areas and years, when all the other factors stay the same. A risk coefficient was calculated comparing different years to year 2004-2005 and different areas to area 1. The significance level was set at $p < 0.05$.

Slaughter batches were divided into three categories, according to their size. Category 0 consisted of slaughter batches with less than 50 reindeer, category 1 of 51-100 reindeer, category 2 of 100-200 reindeer and category 3 of more than 200 reindeer. The division was done by taking quartiles into account. This division of slaughter batches was performed and added to the model for accounting clustering effect of the size of slaughtering batches.

For studying the association between weather and frequency of IIN-s, LOWESS (locally weighted scatterplot smoothing) smoother graphs were developed for visual assessment.

3.4. Availability of data and material

The datasets analyzed during the current study are not publicly available due to including confidential data on diseases of reindeer from reindeer herding cooperatives and abattoirs. The decision of Regional State Administrative Agencies of Lapland Dno. LAAVI/171/03.02.01/2015

4. RESULTS

4.1. Frequency of IIN-s

The overall average frequency of IIN-s in all slaughter batches was 3.5 % (median 0; min 0; max 237.5%). The highest average frequency was in the years 2005-2006 and 2006-2007 with 9.0% (median 0) and 9.1% (median 0), respectively. The lowest average frequency was 0.6% (median 0) during 2012-2013 and 2013-2014. These results are shown in Table 1.

Table 1. Frequency of IIN-s in slaughter batches of reindeers slaughtered in different years in Finland in 2004-2016

Years	Number (%) of slaughter batches	Frequency of IIN[1] %			
		Average	Median	Min	Max
2004-2005	287 (6.9)	5.2	0.0	0.0	125.0
2005-2006	424 (10.1)	9.0	0.2	0.0	100.5
2006-2007	332 (7.9)	9.1	0.0	0.0	90.9
2007-2008	307 (7.3)	1.8	0.0	0.0	90.1
2008-2009	241 (5.8)	1.2	0.0	0.0	60.1
2009-2010	181 (4.3)	1.8	0.0	0.0	150.0
2010-2011	319 (7.6)	1.4	0.0	0.0	36.4
2011-2012	446 (10.7)	0.7	0.0	0.0	51.0
2012-2013	440 (10.5)	0.6	0.0	0.0	75.0
2013-2014	389 (9.3)	0.6	0.0	0.0	50.0
2014-2015	442 (10.6)	6.7	0.0	0.0	237.5
2015-2016	373 (8.9)	3.1	0.0	0.0	100.0
All the years	4181 100.0	3.5	0.0	0.0	237.5

[1]IIN – cases of inflammation indicating necrobacillosis

The within slaughter batch frequency of IIN-s did not follow normal distribution and in 67.4% of the slaughter batches it was 0%. The frequency of IIN-s was 0-10% in 3832 slaughter batches. The distribution is presented on Figure 5.

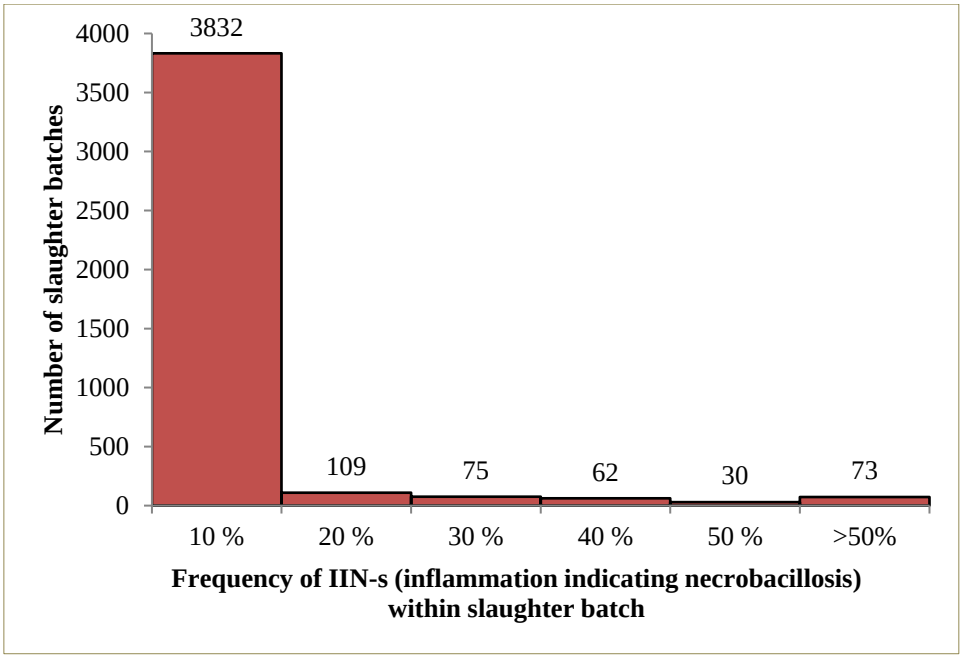


Figure 5. Distribution of within slaughter batch frequencies of IIN-s (inflammation indicating necrobacillosis) in Finnish reindeer during 2004-2016.

The frequency of different inflammations, that were included in the definition of IIN, were 3.4% for pneumonia, pleuritis and peritonitis and 0.1% for abscesses (see Figure 6). The frequency of other inflammations (includes general, skin and other inflammations, pericarditis, arthritis, and stomatitis, excluding IIN findings) during the study period was 0.2%.

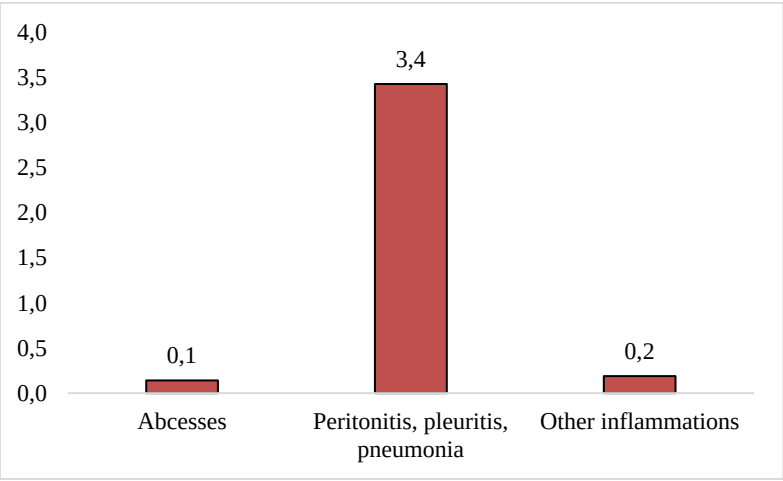


Figure 6. Within batch frequency of different inflammations in Finnish reindeer during 2004-2016

In areal distribution, the highest average frequency of IIN-s (7.7%) was in the area 2 and the lowest (0.4%) in the area 4. The biggest part of the slaughter batches 35.1% (n = 1467) came from area 1, 14.4% (n = 604) from area 2, 25.3% (n = 1059) from area 3 and 25.1% (n = 1051) from area 4 (see Table 2).

Table 2. The frequencies of IIN-s in slaughter batches of reindeers slaughtered in different parts of Finnish reindeer herding area in 2004-2016

Areas	Number (%) of slaughter batches	Frequency of IIN[1] %			
		Average	Median	Min	Max
Area 1	1467 (35.1)	5.3	0.0	0.0	237.5
Area 2	604 (14.4)	7.7	0.0	0.0	125.0
Area 3	1059 (25.3)	1.3	0.0	0.0	100.0
Area 4	1051 (25.1)	0.4	0.0	0.0	90.1

[1]IIN – cases of inflammation indicating necrobacillosis

When comparing slaughter batch categories, the highest frequency (6.8%) was in category 3 and the lowest (1.8%) in category 0. The division between slaughter categories was even – 25.1% (n = 1049) of the batches were smaller than 50 reindeer (category 0), 21.4% (n = 894) between 51-100 reindeer (category 1), 28.0% (n = 1172) between 101-200 reindeer (category 2) and 25.5% (n = 1066) more than 200 reindeer (category 3) (see Table 3).

Table 3. The frequencies of IIN-s according to slaughter batch categories of reindeers slaughtered in different areas of Finnish reindeer herding area during 2004-2016

Slaughter batch category	Number (%) of slaughter batches	Frequency of IIN[1] %			
		Average	Median	Min	Max
0 (1-50 animals) 0	1049 (25.1)	6.8	0.0	0.0	48.3
1 (51-100 animals) 1	894 (21.4)	3.5	0.0	0.0	50.4
2 (101-200 animals) 2	1172 (28.0)	2.4	0.0	0.0	62.5
3 (over 200 animals) 3	1066 (25.5)	1.8	0.0	0.0	237.5

[1]IIN – cases of inflammation indicating necrobacillosis

4.2. Risk factor analysis

Results of the negative binomial model comparing frequencies of IIN between years and areas are shown in Table 4 and Figure 7.

Table 4. Risk coefficients for the inflammations indicating necrobacillosis (IIN) between areas, years and slaughter batch size in Finnish reindeer in 2004-2016

Variable	IRR	95% CI	p value	Wald chi2 p value
Area				<0.001
Area 1	1			
Area 2	1.68	1.15; 2.45	0,007	
Area 3	0.19	0.14; 0.25	<0.001	
Area 4	0.30	0.21; 0.41	<0.001	
Years				<0.001
2004-2005	1			
2005-2006	5.29	3.15; 8.87	<0.001	
2006-2007	3.15	1.83; 5.41	<0.001	
2007-2008	1.52	0.85; 2.72	0,157	
2008-2009	0.71	0.38; 1.33	0,284	
2009-2010	0.45	0.23; 0.85	0,015	
2010-2011	0.33	0.19; 0.58	<0.001	
2011-2012	0.22	0.13; 0.37	<0.001	
2012-2013	0.14	0.08; 0.23	<0.001	
2013-2014	0.15	0.09; 0.27	<0.001	
2014-2015	1.37	0.82; 2.28	0,224	
2015-2016	0.38	0.22; 0.66	0,001	
Slaughter batch size				<0.001
Slaughter category 0	1			
Slaughter category 1	0.47	0.35; 0.65	<0.001	
Slaughter category 2	0.24	0.18; 0.32	<0.001	
Slaughter category 3	0.22	0.15; 0.32	<0.001	

The frequency of IIN was higher in Southern areas (area 1 and 2) compared to the Northern areas (area 3 and 4) where the relative frequencies of IIN-s were 0.19 and 0.30, respectively. This means that the frequency of IIN was 1.7-times bigger in area 2 and approximately 5.3 and 3.33-times smaller in areas 3 and 4, respectively, compared to area 1.

During 2005-2007 the frequency was at the highest (yearly relative frequencies 5.29 and 3.15 compared to 2004-2005 season) and during 2011-2014 at the lowest (yearly relative

frequencies 0.15 – 0.33). During 2014-2015 the frequency got higher again compared to previous 7 years (relative frequency compared to 2004-2005: 1.37), but the result is statistically insignificant as p-level is >0,05. These results show that at its lowest, the frequency was 7-times smaller (in 2012-2013) than in 2004.

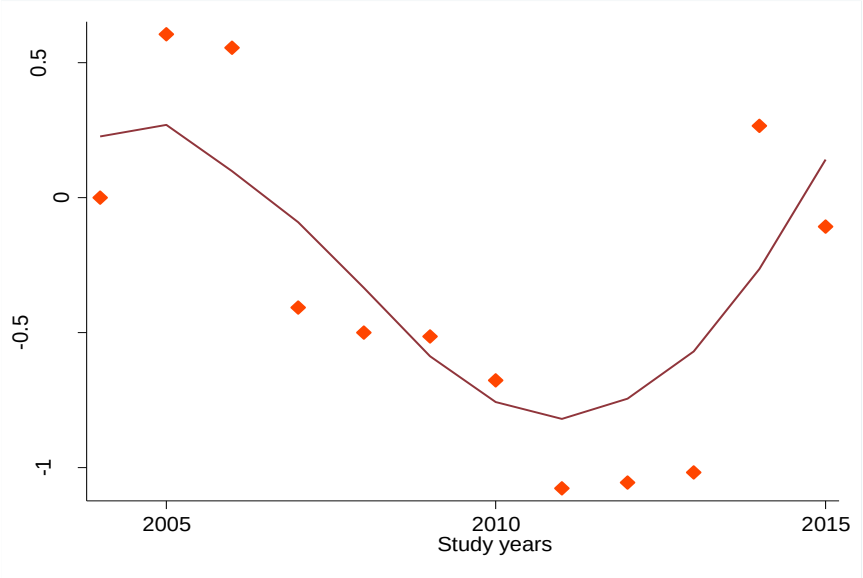


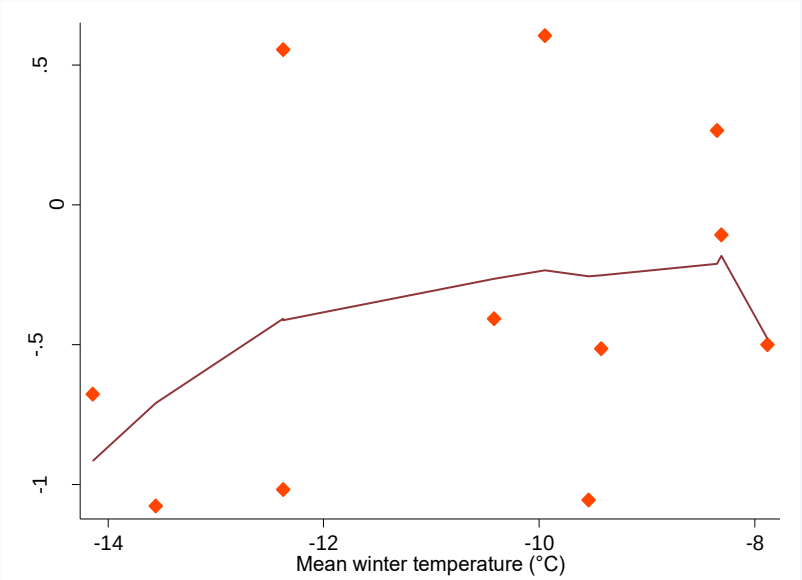
Figure 7. Lowess smoother graph for relative IIN coefficient and study years.

* Relative inflammation indicating necrobacillosis (IIN) coefficients are from random negative binomial model were 2004 was set to zero

4.2. Association between weather and IIN

Mean summer temperatures varied between 7.6ºC (2015-2016) and 14.0ºC (2004-2005) with the average of 12.4ºC in the entire area. Mean winter temperatures stayed between -14.1ºC (2010-2011) and -7.9℃ (2008-2009) average being -10.6ºC. In Utsjoki weather station, which is the Northernmost, mean temperature in summer was 11.6ºC and -11ºC in winter. In Kuusamo, the Southernmost weather station, temperatures were 13,6ºC and -10,6ºC, respectively.

Mean precipitation in the summer was 58.0 mm and 27.3 mm in the winter. In Utsjoki the mean summer precipitation was 53.9 mm and winter 20.5 mm and Kuusamo 59.1 mm and 29.3 mm, respectively. The correlation between weather circumstances and incidence rate ratios is shown on Figure 8 and 9.



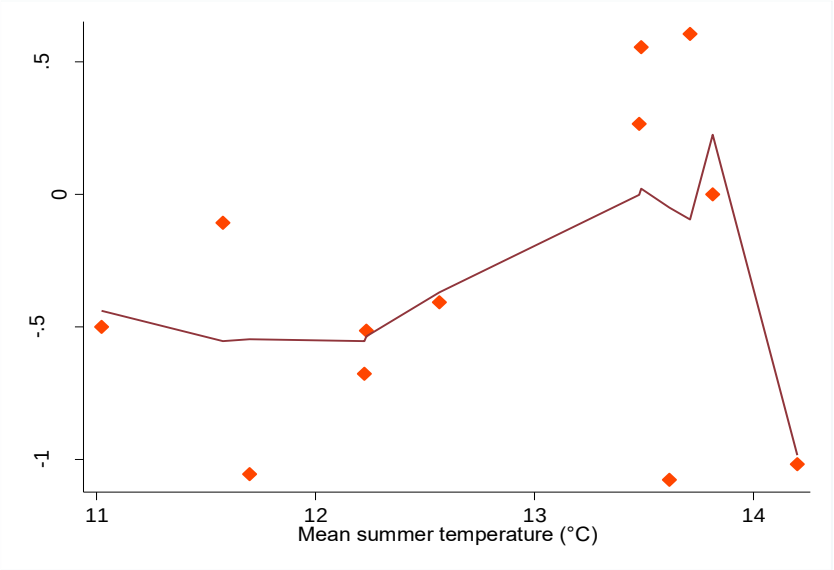


Figure 8. LOWESS smoother graph for relative IIN coefficient and mean winter (above) and summer (below) air temperatures in the Finnish reindeer herding area.

* Relative inflammation indicating necrobacillosis (IIN) coefficients are from random negative binomial model where 2004 was set to zero

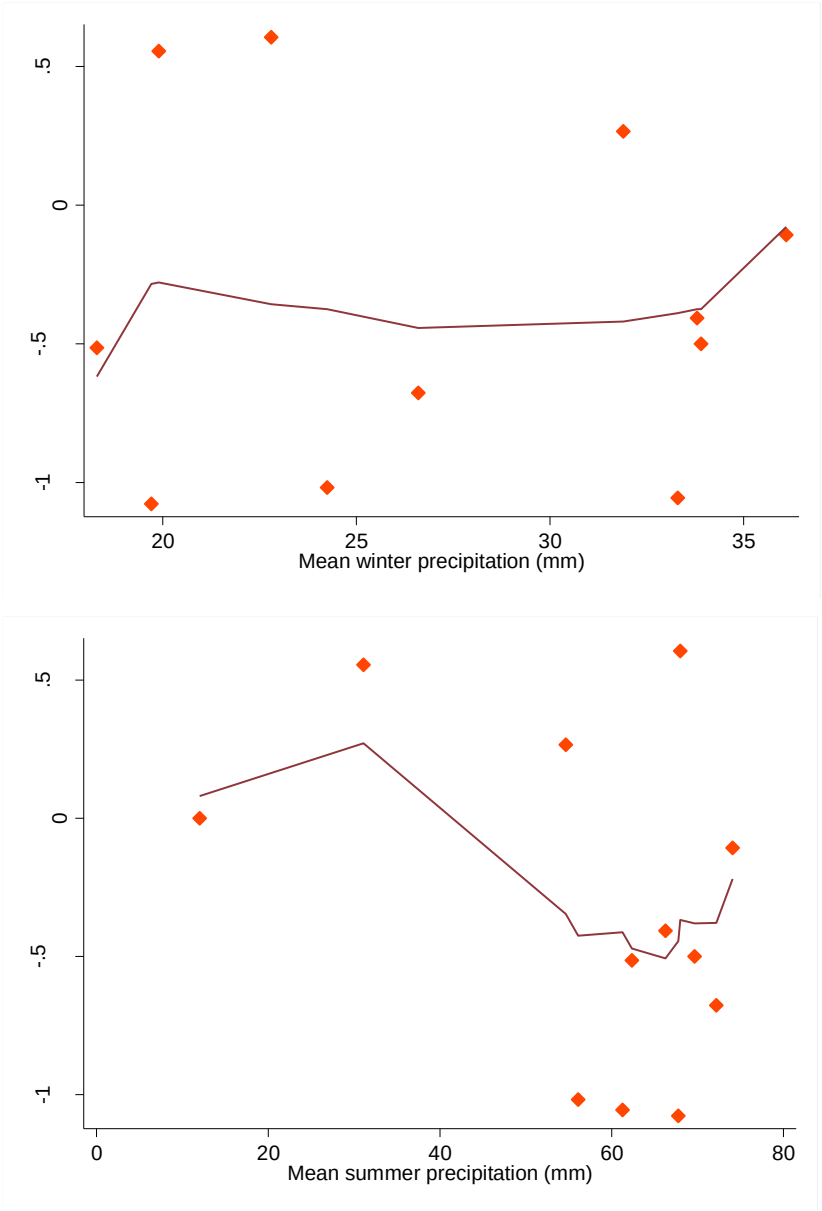


Figure 9. LOWESS smoother graph for relative IIN coefficient and mean winter (above) and summer (below) precipitation in the Finnish reindeer herding area.

* Relative inflammation indicating necrobacillosis (IIN) coefficients are from random negative binomial model were 2004 was set to zero

There was not very clear association between temperatures, precipitation and IIN, but it shows that the risk for IIN might get higher as the mean temperature rises during both winters and summers. When it comes to precipitation, the more it rains in the summer, the lower the risk for IIN. Winter precipitation didn't show any positive correlation.

5. DISCUSSION

This thesis was the first attempt to describe the frequency and dynamics of necrobacillosis in Finnish reindeer. The results show that IIN related inflammations are very common in Finnish slaughter reindeer. This result is in accordance with the earlier published views that IIN is an important reindeer disease (Hirvelä-Koski *et al.* 2017, Laaksonen 2016).

According to the results, there has been outbreak of IIN during 2004-2007 and 2013-2015 of the study period. Summers preceding these high-risk years were warmer (13.1-14.0℃) than the average of the study period (12.4ºC). It was also raining slightly less (37.0 mm in 2004-2006 and 55.4 mm in 2013-2014) than usual (58.0 mm) during these summers. The effect of rain remains unclear, but it might be in association with the temperatures. Although a clearer statistical correlation between temperatures and IIN frequency was expected, this study suggests that higher mean temperatures result in higher frequency of IIN. It's in line with earlier beliefs of the association between weather and necrobacillosis (Nikolaevskii 1961, Haigh and Hudson 1993, Laaksonen, 2016).

Also, the risk for IIN was higher in the Southern areas compared to the Northern areas. Higher mean temperatures during both winters and summers in the South might have had an impact on the incidence of IIN there, although the positive association was not completely clear in this study. The risk for IIN might get higher along climate change, as the mean temperature rises especially in the North.

Supplementary feeding and corralling of reindeer are more common in the South (Maijala and Nieminen 2004), which according to Wikström *et al.* (2014) and Hirvelä-Koski *et al.* (2007) can also predispose to IIN. This is due to stress that animals experience in corals, which makes them more prone to infection and can cause hemorrhages in rumen, that open the port for the bacteria (Rehbinder 1982). Supplementary feeding can cause acidosis in rumen and small wounds in oral cavity, which can lead to necrobacillosis (Laaksonen 2016). Supplementary feeding is getting more common all the time, which might increase the risk of IIN in the future. In the South Finland, outside the reindeer herding area, the reindeer are corralled all year around.

This study was based on the observations and meat inspection findings made by several veterinarians, which may cause bias on the results. All the reindeer meat inspectors have

participated in education for meat inspection and harmonizing meat inspection decisions, and the reporting is simple. The strengths of the study include a long study period, over ten years, and a large number of observations, and the data were collected from official meat inspection decisions of condemnations. Still, it only shows meat inspection findings that indicate necrobacillosis, which are not the exact diagnosis of the disease.

This study suggests that necrobacillosis in Finnish reindeer may be a major disease affecting the health and welfare of the reindeer population, especially in the Southern part of the reindeer herding area. More studies of the drivers of the disease, including better monitoring and sampling, microbiological diagnostic and necropsies, are needed.

CONCLUSIONS

The within batch frequency of IIN was 3.5% during the study period. Highest frequencies were during the years 2005-2007 with 9.0% and 9.1%, respectively. The lowest frequency was 0.6% during 2012-2013 and 2013-2014. Also, negative binomial model showed highest and lowest risk for these years.

Summers preceding high risk years were warmer (13.1-14.0ºC) than the average of the study period (12.4ºC). This indicates that higher mean temperatures result in higher risk of necrobacillosis, which is also suggested in literature before. There was not very clear association between precipitation and IIN, but higher precipitation in the summer showed somewhat lower risk for IIN. Climate change can cause problems in the future when mean temperatures get higher.

The geographical distribution of IIN showed that the within batch frequency (5.3-7.7%) and risk (coefficient) were higher in the Southern areas (areas 1 and 2). Reasons behind this could be higher corralling percentage and supplementary feeding, which both predispose for the disease. Also, higher mean temperature throughout the year can affect the occurrence of necrobacillosis.

This thesis was the first attempt to study the frequency and dynamics of necrobacillosis in Finnish reindeer. The results demonstrated that inflammations, which can be associated to necrobacillosis in general, are important and common disease group and affect reindeer herding, especially in the South. Further studies including better monitoring and sampling, microbiological diagnostic and necropsies, are needed.

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ÜLDKOKKUVÕTE

Nekrobatsilloosile viitavad lihainspektsiooni leiud Soome põhjapõtradel aastatel 2004-2016

Selle töö eesmärk oli selgitada nekrobatsilloosile viitavate põletikuliste muutuste esinemissagedust ja risktegureid Soome põhjapõtradel. Nekrobatsilloos on *Fusobacterium necrophorum*-i tekitatud haigus, mis avaldub abstsesside tekimisega sõravahes või suus ning siseorganites (Laaksonen 2016). Selles töös käsitleti nekrobatsilloosile viitavate põletikena (*inflammation indicating necrobacillosis* – IIN) peritoniiti, pleuriiti, pneumoniat ja abstsesse.

Töös kasutati 4181 tapapartii andmeid, mis hõlmasid 663585 põhjapõdra lihainspektsiooni andmeid Soome põhjapõtrade pidamisalalt aastatest 2004-2016. Käesolevas uuringus loeti aastaks põhjapõtrade karjatamisaasta, mis vältab 1. juunist 31. maini. Iga tapapartii kohta arvutati eelmainitud patoloogiate esinemissagedus (Excel 2016). Riskitegurite analüüsiks kasutati negatiivset binomiaalset mudelit (STATA 14.0), mille väljundiks oli INN-de suhteline sagedus võrdlusrühmades (IRR-*incidence rate ratio*). INN esinemissagedust võrreldi aastate kaupa ning piirkonniti. Soome Meteoroloogia Instituudilt saadud ilmastiku andmetest arvutati keskmine temperatuur ja sademete hulk suvel (juuni-august) ning talvel (detsember-märts) kolmes ilmajaamas – Kuusamo, Sodankylä ja Utsjoki, mis esindavad põhjapõtrade pidamisala lõunast põhja.

Uuringus leiti, et nekrobatsilloosile viitavate põletike keskmine esinemissagedus tapapartiides tervel põhjapõtrade pidamisalal terve uuringuperioodi vältel oli 3,5%. Kõige kõrgem oli esinemissagedus aastatel 2005-2007 (9,0 - 9,1%) ja kõige madalam aastatel 2012-2013 ja 2013-2014 (0,6%).

Ilmastikutingimuste ja IIN esinemissageduse vahel ei olnud väga selget seost, kuid kõrgema riski aastatele eelnevad suved olid soojemad (keskmine temperatuur 13,1-14,0 ^{o}C), kui kogu uuringuperioodil keskmiselt (12,4 ^{o}C). See viitab sellele, et nekrobatsilloosi risk võib olla suurem pärast sooja suve. Sademete ja IIN-i vahel ei olnud selget seost, kuid suurema sademete hulga korral oli IIN-i esinemissagedus mõnevõrra väiksem. Seega võib oletada, et kliima soojenemine tulevikus võib suurendada ka nekrobatsilloosi levikut.

Kõige kõrgem esinemissagedus piirkonniti oli lõunapoolsel põhjapõtrade pidamisalal (5,3-7,7%). See võib olla tingitud sellest, et lõunas peetakse põhjapõtru rohkem koplites ja neile antakse lisasööta. Kõrgemad keskmised temperatuurid võivad ka mõjutada nekrobatsilloosi levikut.

Käesolev uuring on esimene, mis käsitleb nekrobatsilloosi levikut ja risktegureid Soome põhjapõtradel. Tulemustest selgub, et nekrobatsilloosile viitavad põletikud on tähtis haiguste grupp, mis mõjutab põhjapõtrade tervist, eriti lõuna piirkonnas. Selle leviku täpsemaks selgitamiseks on vaja täiendavaid uuringuid, mis põhineksid täpsematel lahanguandmetel ja mikrobioloogilisel diagnoosil.

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